CLAIM + DETAILED DESCRIPTION

[Claim(s)]

[Claim 1] Face performing electrophoresis and a sign is given with photogene with a fluorescent substance with the peculiar excitation wavelength or fluorescence wavelength which is different in a standard sample and a sample sample, respectively, or a luminous wavelength different, respectively. Multi-sign system electrophoresis characterized by performing comparison identification of a standard sample and a sample sample by carrying out electrophoresis of a standard sample and the sample sample simultaneously on the same gel, and detecting the fluorescent substance or photogene by which the sign was carried out to the standard sample and the sample sample from the band obtained after mixing both samples.

[Detailed Description of the Invention]

[0001]

[Industrial Application] This invention relates to the electrophoresis which performs comparison identification of protein, nucleic acid, DNA, etc. quickly and correctly.

[0002]

[Description of the Prior Art] It is separated [in / for the substance which has positive or negative **** / the bottom of an electric field] into the surface like protein by the difference of mobility based on differences, such as a size of the charge of the substance, and molecular weight, etc. Especially gel electrophoresis with which separation is performed in base materials, such as a gel, Since polyacrylamide gel electrophoresis was performed in 1959, much electrophoresis, such as isoelectric focusing, has been developed further and it has been widely used for gel disc electrophoresis, SDS gel disc electrophoresis, and the purpose that separates and analyzes protein, nucleic acid, etc. in genetic engineering.

[0003] As the detection method, when a sample is protein, the staining technique according the gel separated by migration to the Qu Massey brilliant blue, the argentation, etc. is taken. When aimed at DNA, dyeing by the ethidium bromide or the detecting method with a radioisotope (RI) sign is performed. Generally, when a staining technique is used, there are problems, like it is the carcinogen with low (when it is the Qu Massey brilliant blue) sensitivity which spends on a dyeing process and a decolorization process one whole day and night, and requires time for them (in the case of the ethidium bromide). Although there are many cases where electrophoresis of the substance which carried out the sign by RI is being carried out

at the point that sensitivity is high, RI is complicated and there is not only a big problem in respect of safety, but it requires time and expense. When RI is used again, exposing a film also takes time. Therefore, what uses a fluorescent substance as a sign has been reported. It is the kind (adenine (A)) of the end base to the DNA fragment beforehand processed with Sanger method to DNA-sequencing equipment. What attached the separate fluorescent substance corresponding to guanine (G), the thymine (T), and the cytosine (C) is made to migrate on a single migration lane, and there are some which detect the migration pattern of a DNA fragment by receiving the light of each fluorescence wavelength by exciting with laser light.

[0004]

[Problem(s) to be Solved by the Invention] As shown above, by application of electrophoresis, it is (1). The judgment of being that variation has taken place to DNA, and (2) The kind or symptoms mouse of a mouse etc. Mouse which incorporated comparison of DNA, and (3) genes Comparison of DNA etc. is possible. These experiments are conducted by the comparison identification with the zymogram (band) with a standard sample. In electrophoresis, although one way was made to carry out electrophoresis of the gel top, electrophoresis of a standard sample and the sample sample was carried out separately conventionally, and the position of the band obtained from a standard sample and a sample sample, respectively was performing comparison identification. However, the position of the migration band which each gel adjustment does not become the same at all, and is obtained by the variation in distribution of the heat under migration between each lane also in the same gel sometimes produces distortion plentifully, and it causes an error of measurement. Therefore, although measures, such as distorted amendment by cooling of a migration tub and Image Processing Division, are performed, even if it migrates a sample of the same kind, coincidence of a perfect migration image is impossible. Moreover, as for the distorted amendment equipment by above-mentioned Image Processing Division, amendment takes great time very at an expensive price, and the obtained result also has the fault that the picture which was able to take distortion completely is not obtained. For this reason, exact comparison identification and analysis are impossible.

[0005]

[Means for Solving the Problem] In view of the above-mentioned problem, the purpose of this invention is to offer the electrophoresis which does not need the distorted amendment by Image Processing Division. Namely, the fluorescent substance in which this invention has an excitation wavelength which is different, respectively in a standard sample (for example, the normal cell DNA) and a sample sample (for example, the cancer cell DNA), or a fluorescence wavelength, Or on the same gel after giving a sign with photogene with a different luminous wavelength (the marker to A and a sample substance is hereafter called B for the marker to a standard sample) and mixing both samples A from the band simultaneously obtained by performing electrophoresis in a standard sample and a sample sample, and B -- it is the electrophoresis characterized by the thing of a standard sample and a sample sample to do for comparison identification by detecting each signal. In this invention, although the sample of a standard and a sample is described in one case each, each may be plural [two or more]. Even if a standard substance and a sample substance are developed by the same position in the electrophoresis concerning this invention, the excitation wavelength or fluorescence wavelength of a standard substance, Or since a luminous wavelength changes with a standard sample and sample samples, if detection of the standard substance given to each is possible and the signal of only A is acquired from one certain band If it becomes clear that the molecular weight substance is not contained and the signal of A, B, and both is acquired, identification of the molecular weight

substance of a sample sample and a standard sample will be attained, and if the signal of only B is acquired by the sample sample, in it, it will become clear that there is a molecular weight substance which does not exist in a standard substance in a sample sample. In the electrophoresis concerning this invention, since the same gel and the same lane perform electrophoresis for both samples (a standard sample and sample sample) simultaneously, the ideal electrophoresis which does not receive which distorted influence of the spot accompanying gel adjustment can be constituted. Since the analysis of an abnormality of the genes can carry out in high precision easily if this invention is used, research of an abnormality of the genes can be developed by leaps and bounds.

[0006] A fluorescent substance, photogene, etc. are used as a marker. When a fluorescent substance is used, the substance with which an excitation wavelength differs from a fluorescence wavelength must be used for the markers A and B. As an example, some combination of a fluorescent substance is shown in Table 1. The inside of Table 1, (lambdaex expresses an excitation wavelength among Table 1, and lambdaem expresses a fluorescence wavelength.) making the excitation light side the same in (1) and (2) (setting near the excitation wavelength of 495nm, and to (2) in (1) -- the with an excitation wavelength of 325nm light source or (light source + interference filter) use, and fluorescence light-receiving side -- A and B -- the interference filter of each fluorescence wavelength being prepared.) (3) (4) setting and making the fluorescence light-receiving side the same conversely (the interference filter of per 578nm being installed in front of a photo acceptance unit in the interference filter of per 520nm, and (4) in (3)) -- the excitation light side -- A and B -- the interference filter of each excitation wavelength is prepared. And replace each interference filter by turns, it is made to synchronize with it, and the signal is taken. What is necessary is to choose what has the narrow half width of an interference filter, or just to choose the big combination of the difference of the excitation wavelength or fluorescence wavelength of A and B, or a luminous wavelength, in order to raise S/N. In the method of this invention, [the standard substances A and B] By choosing not the thing restricted to one kind, respectively but what has the small half width of an interference filter That is, by separating and measuring each wavelength, by choosing two or more kinds of markers A and B, respectively about two or more kinds of standard samples and sample samples, electrophoresis can be simultaneously performed on the same gel and comparison identification can be carried out for each sample. Although a work example explains the method of this invention in more detail below, this invention is not limited to these indications.

[Table 1]

	標識物質A	標識物質B
(1)	ミロシアニン540 λ_{ex} =500nm λ_{em} =572nm	FITC λ_{ex} =490nm λ_{em} =520nm
(2)	4-ACETAMID-4' -MALEIMIDYLSTILBENE-DISULFONIC ACID λ_{ex} =325nm λ_{em} =404nm	DIDANSYL -L-CYSTINE λ_{ex} =328nm λ_{em} =545nm
(3)	FLUORESCEIN-5-MALEIMIDE λ_{ex} =495nm λ_{em} =520nm	1. 5-IAEDANS λ_{ex} =337nm λ_{em} =520nm
(4)	MRITC λ_{ex} =540nm λ_{em} =577nm	DANSYL CHLORIDE λ_{ex} =340nm λ_{em} =578nm

[0007]

[Work example 1] In figure .1, the light which came out of argon laser 1 is bent by a mirror 2, and irradiates a migration board top. A mirror moves so that laser light may scan an electrophoresis board top by a motor. In addition, the band of the standard sample from which electrophoresis was already performed and the electrophoresis board was separated by migration on the board, and a sample sample exists.

[0008] A normal rat (henceforth X) and the serum obtained from the rat for the 2nd week (it is called Following Y) after transplanting a rhodamine sarcoma as a sample sample were used as a standard sample. In the protein in the serum from Y, FITC was labeled for merocyanine 540 with the respectively well-known procedure at the protein in the serum from X. The polyacrylamide gel performed electrophoresis. Gel concentration is 10% - 20% of straight line acrylamide concentration gradient gel, and performed 20mA (17 hours) energization. As buffer solution for migration, it is 0.025. Mole Tris-0.192 Morque ricin was used. It is condensed with a lens 4 and the fluorescence generated from each fluorescent substance is put in to the interference filter unit 5. The interference filter unit 5 is 570nm. 520nm It has the mechanism which an interference filter holder rotates by a motor, and the merocyanine 540 which is a sign fluorescent substance,

and FITC can be detected now by turns. The fluorescence which passed along the interference filter unit enters into the photomultiplier tube 6 which is a detector, and is detected. This signal is processed synchronizing with the change of the filter of an interference filter unit, and the thing from the serum from X and the thing from the serum from Y is judged. Two signals about each marker simultaneously obtained from the same gel were able to be compared, the migration position of the sample sample about the standard sample on a gel was able to be checked, and both comparison identification was able to be performed.

[0009]

[Work example 2] A standard and a sample sample are processed with 6 base recognition restriction enzyme. Both samples were mixed and electrophoresis was performed, after reacting a merocyanine 540 sign nucleotide to a standard sample and making an FITC sign nucleotide react to a sample sample, respectively. 3 is using the 8% polyacrylamide gel which contains 7mol urea with the slab-gel-electrophoresis board of a vertical mold. TBE buffer solution was used as buffer solution.

[0010] It is condensed with a lens 4 and the fluorescence generated from each fluorescent substance is put in to the interference filter unit 5. The interference filter unit 5 is equipped with the mechanism which an interference filter holder (570nm and 520nm) rotates by a motor, and can detect now by turns the merocyanine 540 and FITC which are a sign fluorescent substance. The fluorescence which passed along the interference filter unit enters into the photomultiplier tube 6 which is a detector, and is detected. It is processed synchronizing with the change of the filter of an interference filter unit, and this signal is the thing and sample sample from a standard sample DNA fragment. The thing from a DNA fragment is judged. Thus, two signals about each marker obtained from the same gel were able to be compared, the migration position of the sample sample about the standard sample on a gel was able to be checked, and both comparison identification was able to be performed. Since the difference in the migration position of a standard sample and a sample sample was what is produced by difference of the DNA sequence of a gene, it has analyzed an abnormality of the genes by detecting this difference.

[0011]

[Effect of the Invention] In the electrophoresis of this invention, comparison identification is possible in high precision, without receiving the distorted influence of the position of the spot of electrophoresis, since electrophoresis of the same gel top is simultaneously carried out for a standard sample and a sample sample.

[Translation done.]